5. SUMMARY

In the present investigation, the efficacy of ethanolic leaf extract of the ornamental and medicinal plant, Cassia fistula Linn. was evaluated for its hepatoprotective and antioxidant properties against carbon tetrachloride (CCl₄), Isoniazid (INH) and antitubercular drugs (INH+RIF+PYR) induced hepatotoxicity in rats.

Clean dried leaves of Cassia fistula Linn. (Family: Caesalpinaceae) were soaked in 90 percent ethanol for extraction of active principles. The filtered leaf extract was concentrated to dryness at 60°C. The yield of ethanolic leaf extract (ELE) was 19 to 20 percent. On phytochemical screening, the ELE showed positive results for glycosides, flavonoids, saponins, tannins and alkaloids.

Acute toxicity test showed nil mortality in rats treated with ELE (at concentrations of 250, 500, 1000, 1500, 2000 and 2500 mg/kg) upto 72 h. Further, physiological, neurological and behavioral abnormalities were absent. ELE administration was deemed safe and a dose of 500 mg/kg was chosen for the evaluation of hepatoprotective and antioxidant effects in rats against the above specified hepatotoxicants induced hepatotoxicity in rats.

In a preliminary study, ELE’s hepatoprotective property against sub-acute CCl₄ induced hepatocellular damage was investigated. Simultaneous treatment of ELE with CCl₄ showed marked hepatoprotective property as indicated by complete prevention of CCl₄ induced increase in
marker enzymes of liver toxicity (AST, ALT, ALP, LDH and γ-GT) in serum accompanied by a decrease in these parameters in liver tissue. This treatment also completely prevented the CCl₄ induced increase in LPO and decrease in SOD and CAT, indicating its hepatoprotective and antioxidant properties.

CCl₄ induced histopathological changes manifested as hepatocellular necrosis, fatty degeneration, excessive vacuolation and mild sinusoidal dilation of the liver tissue were also restored towards normalcy on simultaneous administration of ELE with CCl₄, indicating hepatoprotective property of ELE against CCl₄ induced hepatotoxicity.

It is likely that CCl₄ induce hepatotoxicity by virtue of its ability to form trichloromethyl radical and hydroperoxyl radical, resulting in hepatocellular membrane instability. ELE might quench these free radicals and thus protect the liver against CCl₄ induced hepatocellular damage.

Since hepatoprotective and antioxidant properties of ELE, on its simultaneous treatment against CCl₄ induced hepatotoxicity was evident, it was of interest to evaluate the same against INH alone and a combination of antitubercular drugs (INH+RIF+PYR) induced hepatotoxicity in rats. In this study, *Silymarin* (SIL) was used as a standard hepatoprotective and antioxidant agent for comparison with the efficacy of ELE.

Administration of INH alone (50 mg/kg b.w., i.p.) for 30 days caused an elevation in the status of marker enzymes of hepatotoxicity (AST, ALT, ALP, LDH, and γ-GT) in serum, accompanied by a decrease in their status in the liver tissue, indicating onset of hepatocellular damage.
This treatment also caused an elevation in lipid peroxidation (LPO) accompanied by a decrease in the levels of enzymic antioxidants (GPx, GST, GR, SOD and CAT) and non-enzymic antioxidants (GST, Vit.C and Vit.E) in the liver tissue of rats, indicating oxidative stress and disruption of antioxidant defence mechanism. The hepatocellular membrane instability induced by INH was also evident by the decrease in the status of membrane bound ATPases (Na⁺/K⁺, Ca²⁺ and Mg²⁺) in the liver tissue.

Hyperlipidemia, induced by INH alone treatment was also evident by an increase in the levels of lipid parameters (TL, TG, CHO, PL and FFA) in the liver tissue. In addition, INH administration caused an increase in the levels of lipid parameters (TL, TG, CHO and FFA) in the liver tissue accompanied by a decrease in their levels in adipose tissue. In the liver tissue, however, this treatment caused a decrease in the levels of PL.

The increase in the status of lipid parameters in liver tissue indicates the onset of hepatic steatosis and the decrease in their levels in adipose tissue denotes mobilization of depot fat. The mobilization of lipids from depot fats cause elevation in the concentrations of lipid in the liver, resulting in hepatic steatosis and hyperlipidemia are plasma. INH induced hepatic steatosis and hyperlipidemia is thus evident. Hepatocellular damage and hepatic steatosis were also evident in the histopathological studies of liver tissue of rats administered INH.

Simultaneous treatments of ELE as well as SIL, protected the liver against INH induced changes in the status of marker enzymes in serum and
liver tissue, enzymic and non-enzymic antioxidants, ATPases in liver tissue. These treatments also prevented the increase in the levels of lipid parameters in liver tissue and fall in their levels in adipose tissue. These results indicate the hepatoprotective, antioxidant and antilipidemic properties of ELE and SIL against INH induced hepatotoxicity.

Administration of antitubercular drugs (INH – 25 mg/kg; RIF – 50 mg/kg; and PYR – 140 mg/kg b.w., i.p.) for 45 days produced hepatotoxicity as indicated by an increase in the status of marker enzymes of hepatotoxicity in serum, accompanied by a decrease in their levels in the liver tissue. This treatment also caused an increase in oxidative stress and disruption of antioxidant defence system, as indicated by increase in LPO accompanied by a decrease in the status of enzymic and non-enzymic antioxidants and ATPases in the liver tissue.

Hyperlipidemia and hepatic steatosis induced by antitubercular drugs administration were also evident by an increase in the status of lipid parameters in plasma and liver tissue, accompanied by a decrease in their level in adipose tissue. Hepatic steatosis and necrosis induced by administration of antitubercular drugs were also evident in histopathological studies.

As compared to INH alone treatment, administration of antitubercular drugs exhibits a more pronounced hepatocellular damage and hepatic steatosis.
It is believed that toxic metabolites of INH i.e., acetylhydrazine and hydrazine and their reactive radicals released during the metabolism, mediated by cytochrome P450 enzymes, are the cause of INH induced hepatotoxicity. It is likely that the combined treatment of antitubercular drugs might exhibit more pronounced hepatotoxic potential due to the enzyme inducing property of RIF and/or to the hepatotoxic metabolite of PYR i.e., pyrazinoic acid.

The acetylcarbazonium ions and acetyl free radicals released during metabolism of acetylhydrazine and free radicals generated during metabolism of hydrazine might induce oxidative stress leading to hepatocellular membrane instability, resulting in hepatocellular damage. The precise mechanism of INH and antitubercular drugs induced hepatotoxicity, however, is not clearly established and further studies are warranted on these lines.

Hepatic steatosis induced by INH and antitubercular drugs denotes disruption of lipid metabolism in plasma, liver and adipose tissues. It is likely that steatosis would have been caused due to increased mobilization of free fatty acids from the adipose tissues into the liver, increased TG synthesis in the liver or decreased secretion of lipoproteins.

Simultaneous treatment of ELE as well as SIL protected the liver against all the above antitubercular drugs induced hepatotoxicity, indicating their hepatoprotective, antioxidant and antilipidemic properties.
Simultaneous treatment of SIL shows better hepatoprotective, antioxidant and antilipidemic properties both against INH alone and antitubercular drugs induced hepatotoxicity and hepatic steatosis as compared to simultaneous treatment with ELE.

SIL, a flavonolignan, might inhibit the oxidative stress and maintain membrane stability against hepatotoxicity induced by antitubercular drugs and INH, and thus exhibit hepatoprotective and antioxidant effect. The active principles (flavonoids, glycosides, tannins, saponins and alkaloids) present in ELE might exhibit hepatoprotective, antioxidant and antilipidemic properties by inhibiting oxidative stress and maintenance of membrane stability induced by INH and antitubercular drugs. The precise mechanism by which these agents inhibit oxidative stress induced by antitubercular drugs and INH needs to be elucidated.

In conclusion, simultaneous treatment of ELE and SIL exhibits hepatoprotective and antioxidant properties against INH alone and antitubercular drugs induced hepatotoxicity in rats. This beneficial effect would have been brought about by the inhibition of oxidative stress and maintenance of hepatocellular membrane stability. The antilipidemic property of these principles against the above toxin induced hyperlipidemia and steatosis would have been brought about by the restoration of impaired lipid metabolism in liver and adipose tissues.